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(71) Applicant: PROTOS CORPORATION [US/US]; 4560 Horton Street, Emeryville, CA 94608 (US).

(71)(72) Applicant and Inventor: KOMPIS, Ivan [CH/CH]; CH-4002 Basel (CH).

(72) Inventors: BLANEY, Jeffrey, M. ; 6601 Saroni Drive, Oakland, CA 94611 (US). MARLOW, Charles, K. ; 765 8th Avenue, San Francisco, CA 94131 (US).

(74) Agents: ROBINS, Roberta, L. et al.; Morrison & Foerster, 545 Middlefield Road, Suite 200, Menlo Park, CA 94025 (US).

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(54) Title: SPECIFIC INHIBITION OF DIHYDROFOLATE REDUCTASE AND COMPOUNDS THEREFOR

(57) Abstract

Compounds derived from pyrimidines having improved activity against fungi such as *Pneumocystis carinii*, and improved selectivity for *P. carinii* dihydrofolate reductase over human dihydrofolate reductase, are disclosed. *P. carinii* pneumonia is advantageously treated with the disclosed compounds.

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SPECIFIC INHIBITION OF DIHYDROFOLATE REDUCTASE AND COMPOUNDS THEREFOR

Description

Technical Field

This invention relates to pharmacology and the inhibition of enzymes specific to pathogens. More particularly, the invention relates to methods for specifically inhibiting the enzyme dihydrofolate reductase in fungal pathogens, and compounds therefor.

Background of the Invention

Pneumocystis carinii pneumonia (PCP) is a significant, life-threatening infection in immunocompromised subjects, and is a leading cause of morbidity and mortality in patients presenting acquired immunodeficiency syndrome (AIDS). Since the onset of the AIDS epidemic, the incidence of PCP has risen from approximately 200 cases per year to more than 25,000 cases per year in the United States.

Due to the lack of a continuous in vitro culture system, and the cumbersome nature of the rat model of PCP, anti-P. carinii therapy has been developed largely on the assumption that P. carinii was a species of protozoa, and thus that anti-protozoal agents were likely to be effective. The two principle therapeutic modalities, trimethoprim/sulfamethoxazole and pentamidine, were developed empirically. However, P. carinii has recently been suggested to belong instead to the Kingdom Fungi (J.C. Edman et al., Nature (1988) 334:519-22).

Trimethoprim (U.S. Pat. No. 2,909,522) and pyrimethamine, and other dihydrofolate reductase (DHFR) inhibitors are known to be effective anti-bacterial, and anti-protozoal agents due to the central role played by DHFR in the metabolic synthesis of nucleic acid precursors. Despite their efficacy when used in conjunction with a sulfonamide, trimethoprim and pyrimethamine are alone poor inhibitors of P. carinii DHFR. For example, trimethoprim and pyrimethamine exhibit 50% inhibition concentrations (IC_{50}) of 8 and 2,500 nM for E. coli DHFR, while IC_{50}s for P. carinii DHFR are 39,600 and 2,400 nM, respectively. Other anti-folates have been shown to be more effective inhibitors of P. carinii DHFR, but require concomitant administration of leucovorin to prevent toxicity to the host. Allegra et al. (U.S. Pat. No. 4,694,007) suggested treatment of P. carinii and Toxoplasmosis gondii with 2,4-diamino-5-methyl-6-[(3,4,5-trimethoxyanilino)-methyl]quinazoline (trimetrexate), on the theory that the DHFR enzyme in this pathogens is more similar to mammalian DHFR than to prokaryotic DHFR.

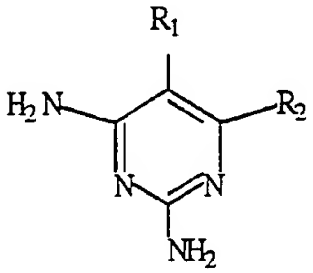
Prior to the AIDS epidemic, these types of agents were sufficient for treatment of the rare cases of P. carinii pneumonia. However, in the HIV-positive patient, therapy and prophylaxis with the standard anti-P. carinii agents are complicated by frequent toxic and allergic side effects. New compounds that surpass the efficacy of the known antifolates in treating PCP are desirable, especially inhibitors having greater selectivity for P. carinii DHFR relative to the host (particularly human) DHFR than known inhibitors such as trimethoprim.

Commonly-owned copending U.S. Patent Application Serial No. 447,181, filed 7 December 1989 disclosed several DHFR inhibitors exhibiting good selectivity for P. carinii DHFR.

Disclosure of the Invention

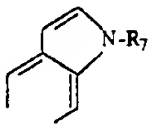
We have now found compounds which exhibit superior activity against DHFR from fungal pathogens, such as P. carinii, and which exhibit much higher selectivity for the fungal enzyme as compared with the mammalian (human) enzyme. These compounds are represented generally by Formula I:

Formula I:



wherein

R_1 is 3-R_3-4-R_4-5-R_5-benzyl or (N-R_6)-8-azabicyclo[3.2.1]oct-3-yl; and

R_2 is H; or R_1 and R_2 together form  where R_3 and R_5 are

independently selected from the group consisting of H, lower alkoxy, lower alkylthio, lower alkylsulfinyl, vinyl, carboxy-lower alkyl, carboxy-lower alkoxy, dicarboxy-lower alkyl, dicarboxy-lower alkoxy, aryl-lower alkoxy, arylsulfonyl-lower alkoxy, arylsulfamido-lower alkoxy, and radicals of formula $-O(CH_2)_n-COR_8$, where n is an integer from 0 to 6 and R_8 is an amino acid; R_4 is selected from the group consisting of lower alkoxy, aryl-lower alkoxy, lower alkylthio, halo, lower alkenyl, lower alkenyloxy, and pyrrolyl; with the proviso

that R_3, R_4, and R_5 are not simultaneously methoxy; R_6 is selected from the group consisting of unsubstituted aryl and aryl substituted with one to three radicals selected from the group consisting of halo, lower alkyl, lower alkoxy, lower alkylthio, carboxy, carbamido, carboxy-lower alkyl, and carbamido-lower alkyl; and R_7 is selected from the group consisting of aryl and aryl-lower alkyl, where aryl may be substituted with one to three radicals selected from the group consisting of halo, lower alkyl, lower alkoxy, lower alkylthio, carboxy, carbamido, carboxy-lower alkyl, and carbamido-lower alkyl; and lower alkyl esters, amides thereof, and pharmaceutically acceptable addition salts.

One aspect of the invention is a method for treating a fungal infection (such as P. carinii) in a mammal by administering an effective amount of a compound of formula I.

Another aspect of the invention is a composition for treating a fungal infection (such as P. carinii) in a mammal comprising an effective amount of a compound of formula I in combination with a pharmaceutically acceptable excipient.

Another aspect of the invention is the use of a compound of formula I to prepare a composition for treating a fungal infection (such as P. carinii) in a mammal comprising an effective amount of a compound of formula I in combination with a pharmaceutically acceptable excipient.

Modes of Carrying Out The Invention

A. Definitions

The terms "fungal infection" and "fungal pathogen" refer to the infection of a mammal with an organism of the Kingdom Fungi, for example Pneumocystis carinii, Aspergillus, Candida, Fusarium, and the like. The presently preferred method of the invention is the treatment of Pneumocystis carinii using the compounds of the invention.

The term "pharmaceutically acceptable" refers to compounds and compositions which may be administered to mammals without undue toxicity. Exemplary pharmaceutically acceptable salts include mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like.

The term "effective amount" refers to an amount of compound sufficient to exhibit a detectable therapeutic effect. The therapeutic effect may include, for example, without limitation, inhibiting the growth of pathogens, inhibiting or preventing the release of toxins by pathogens, killing pathogens, and preventing the establishment of infection (prophylaxis). The precise effective amount for a subject will depend upon the subject's size and health, the nature of the pathogen, the severity of the infection, and the like. Thus, it is not possible to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by routine experimentation based on the information provided herein.

The term "lower alkyl" refers to saturated straight or branched-chain radicals consisting of carbon and hydrogen having from 1 to 6 carbon atoms, inclusive, such as methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, n-hexyl, and the like. "Lower alkoxy" refers to a radical of the form R-O-, where "R" is lower alkyl as defined above. Suitable examples include methoxy, ethoxy, propoxy, butoxy, and the like. Similarly, "lower alkylthio" refers to radicals of the form R-S-, and "lower alkylsulfinyl" refers to groups of the form R-S(=O)-. For example, one may employ methylthio, ethylthio, methylsulfinyl, t-butylsulfinyl, and the like. "Lower alkenyl" refers to straight or branched-chain radicals consisting of carbon and hydrogen having 2-6 carbon atoms and at least one double bond between a pair of carbon atoms, such as ethenyl (vinyl), 2-propenyl, 1-methylethenyl, 2-butenyl, 3-butenyl, and the like.

The term "carboxy-lower alkyl" refers to radicals having the form $-(CH_2)_n-COOH$, where n is an integer from 1 to 6 inclusive. "Dicarboxy-lower alkyl" indicates lower alkyl chains having two COOH groups attached.

"Aryl" denotes cyclic hydrocarbon radicals of 6-10 carbon atoms which exhibit aromatic character, for example phenyl and napthyl.

The term "halo" refers to fluoro, chloro, bromo, and iodo. The term "amino acid" refers to any of the 20 or so commonly occurring amino acids, for example, glycine, alanine, arginine, phenylalanine, glutamic acid, valine, histidine, proline, ornithine, norleucine, and the like. When attached as R_8 in a radical of the form $-O(CH_2)_n-COR_8$, the amino acid will preferably be attached via a peptide bond, i.e. by a bond between the amino group of the amino acid and the acyl carbon of the radical.

The term "coadministering" means administration of a compound of the invention in combination with a second therapeutic agent. The second therapeutic agent is a dihydropteroate synthase inhibitor, preferably dapsone or a sulfa drug. Suitable sulfa drugs include, without limitation, sulfadiazine, sulfamethoxazole, and the like. Coadministration may be simultaneous, for example by administering a mixture of the therapeutic agents, or may be accomplished by administration of the agents separately within a short time period.

B. General Method

The compounds of the invention are structurally related to the compound trimethoprim (2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine), the synthesis for which is known in the art. See for example U.S. Pat. No. 2,909,522, which describes the synthesis of trimethoprim and related compounds. Compounds of formula I may similarly be synthesized by those of ordinary skill in the art. Syntheses of such compounds are described in the following U.S. patents: Hitchings et al. (2,658,897); Hitchings et al. (2,909,522); Hoffer (3,341,541); Roth (3,772,289); Roth et al. (3,819,629); Roth (3,822,264); Kompis et al.

(3,931,181); Herrling (3,980,649); Liebenow et al. (3,992,379); Kompis (4,024,145); Rosen (4,033,962); Kompis et al. (4,039,543); Jernow et al. (4,075,209); Perun et al. (4,087,528); Fritschi et al. (4,180,578); Kompis et al. (4,203,980); Poe et al. (4,258,045); Daluge et al. (4,438,267); Dall'Asta (4,485,248); Kompis et al. (4,515,948); Swaringen et al. (4,568,744); Roth et al. (4,587,341); Kompis et al. (4,590,270); Daluge et al. (4,603,136); Kompis et al. (4,792,557); and Seydel et al. (4,912,112).

Presently preferred compounds of the invention are:

2,4-diamino-5-[3,5-dimethoxy-4-(2-hydroxyprop-2-yl)benzyl]pyrimidine;

2,4-diamino-5-(3,5-dimethoxy-4-N-pyrrolylbenzyl)pyrimidine;

2,4-diamino-5-(3,5-diethoxy-4-N-pyrrolylbenzyl)pyrimidine;

2,4-diamino-5-(3,5-dipropoxy-4-N-pyrrolylbenzyl)pyrimidine;

2,4-diamino-5-(3,5-dibutoxy-4-N-pyrrolylbenzyl)pyrimidine;

2,4-diamino-5-(3,5-diethoxy-4-carboethoxybenzyl)pyrimidine;

2,4-diamino-5-(3,5-divinyl-4-vinyloxybenzyl)pyrimidine;

2,4-diamino-5-[3-(4-N-acetaminophenyl)sulfonaminoethoxy-4,5-dimethoxybenzyl]-pyrimidine;

2,4-diamino-5-[3-(4-aminophenyl)sulfonaminoethoxy-4-methoxybenzyl]pyrimidine;

2,4-diamino-5-[3-(4-N-acetaminophenyl)sulfonaminoethoxy-4-bromo-5-methoxybenzyl]-pyrimidine;

2,4-diamino-5-[3-(4-aminophenyl)sulfonaminoethoxy-4-bromo-5-methoxybenzyl]-pyrimidine;

2,4-diamino-5-(3-methoxy-4,5-dibenzyloxybenzyl)pyrimidine;

2,4-diamino-5-(4-benzyloxybenzyl)pyrimidine;

2,4-diamino-5-(3,4-dibenzyloxy-5-methoxybenzyl)pyrimidine;

2,4-diamino-5-(3,4-dimethoxy-5-benzyloxybenzyl)pyrimidine;

2,4-diamino-5-[3,5-diethoxy-4-(propen-2-yl)benzyl]pyrimidine;

2,4-diamino-5-[3,5-dimethoxy-4-(propen-2-yl)benzyl]pyrimidine;

2,4-diamino-5-(3,5-dimethoxy-4-methylthiobenzyl)pyrimidine;

2,4-diamino-5-(3-methylsulfinyl-4-methoxy-5-methylthiobenzyl)pyrimidine;

2,4-diamino-5-[3-(4,6-dicarboxyhexyloxy)-4-bromo-5-methoxybenzyl]pyrimidine;

2,4-diamino-5-[3-(3-carboxymethylamino-3-oxopropoxy)-4-methoxybenzyl]-pyrimidine;

2,4-diamino-5-(3-[3-(1,3-dicarboxypropyl)amino-3-oxopropoxy]-4-bromo-5-methoxy-benzyl)pyrimidine;

2,4-diamino-5-[3,5-dimethoxy-4-((2-phenylsulfinyl)acetyl)benzyl]pyrimidine;

2,4-diamino-5-[3-amino-4-methyl-5-(N-pyrrolyl)benzyl]pyrimidine;

2,4-diamino-5-(3,5-di-N-pyrrolyl-4-methoxybenzyl)pyrimidine;

2,4-diamino-5-[3,5-di-methoxy-4-(3-hydrocarboxy-1-oxopropylamino)benzyl]pyrimidine;

2,4-diamino-5-[3,5-dimethoxy-(4-acetaminophenylsulfonamino)benzyl]pyrimidine;

2,4-diamino-5-(3,5-dimethoxy-4-propylbenzyl)pyrimidine;

2,4-diamino-5-(3,5-dichloro-4-N-pyrrolylbenzyl)pyrimidine;

2,4-diamino-5-[3,5-dimethoxy-4-(2-(2-(2-methoxy)ethoxy)ethoxy)ethoxybenzyl]-pyrimidine;

2,4-diamino-5-[3-(3-benzyloxycarbonylmethylamino-3-oxopropoxy)-4-bromo-5-methoxy-benzyl]pyrimidine;

2,4-diamino-5-[3-(3-carboxymethylamino-3-oxopropoxy)-4-bromo-5-methoxybenzyl]-pyrimidine;

2,4-diamino-5-[3-methoxy-4-bromo-5-(4-methylaminobenzamidoethoxy)benzyl]-pyrimidine;

3-(2,4-diaminopyrimidin-5-ylmethyl)-8-(3,5-dimethoxyphenyl)-8-azabicyclo[3.2.1]octane;

2H,3H-dihydro-5-(2,4-diaminopyrimidin-5-ylmethyl)-6,7-dimethoxybenzofuran;

5-(2,4-diaminopyrimidin-5-ylmethyl)-7-methoxy-8-bromo-1,2-benzopyran;

5-(2,4-diaminopyrimidin-5-ylmethyl)-7,8-dimethoxy-1,2-benzopyran;

2,4-diamino-5-[3-phenyl-5-(3-methoxypropoxy)benzyl]pyrimidine;

2,4-diamino-7-(3,5-dimethoxybenzyl)pyrrolo[2,3-f]quinazoline;

2,4-diamino-5-[6-(4-methoxybutoxy)naphth-1-yl]pyrimidine;

2,4-diamino-5-(2,7-dimethylbenzpyrazol-5-ylmethyl)pyrimidine;

2,4-diamino-5-(4,5,6-trimethoxy-2,3-dihydroinden-1-yl)pyrimidine; and

2,2-dimethyl-5-(2,4-diaminopyrimidin-5-ylmethyl)-7-methoxybenz[b]dioxolane.

The most preferred compounds at present are 2,4-diamino-5-(3,5-diethoxy-4-N-pyrrolylbenzyl)pyrimidine and 2,4-diamino-5-[3,5-dimethoxy-4-(2-hydroxyprop-2-yl)benzyl]pyrimidine.

Compositions of the invention for administration will generally include an effective amount of a compound of formula I in addition to a pharmaceutically acceptable excipient. Suitable excipients include most carriers approved for oral or parenteral administration, including water, saline, Ringer's solution, Hank's solution, and solutions of glucose, lactose, dextrose, ethanol, glycerol, albumin, and the like. These compositions may optionally include stabilizers, antioxidants, antimicrobials, preservatives, buffering agents, surfactants, and other accessory additives. A presently preferred vehicle comprises about 1 mg/mL serum albumin in phosphate-buffered saline (PBS). A thorough discussion of suitable vehicles for parenteral administration may be found in E.W. Martin, "Remington's Pharmaceutical Sciences" (Mack Pub. Co., current edition).

The precise dosage necessary will vary with the age, size, and condition of the subject, the nature and severity of the disorder to be treated, and the like: thus, a precise effective amount cannot be specified in advance. However, appropriate amounts may be determined by routine experimentation with animal models. In general terms, an effective dose of compound of formula I will range from about 10 µg/Kg to about 50 mg/Kg. Suitable animal models include the mouse model illustrated in the Examples below. Rats and other rodents have DHFR very similar to the human enzyme, and thus make suitable animal models. A group of experimental animals is inoculated with 10-100 LD_{50}s of Pneumocystis carinii, followed by treatment with a solution of test compound. A negative control group is left untreated, while a positive control group is treated with a standard therapy, such as trimethoprim. Administration of the compounds is prefer-

ably per os (e.g., using a gavage), but may be parenteral, for example by subcutaneous or intramuscular injection, or by inhalation of an aerosol. The animals are monitored during treatment, and are sacrificed and examined after 60 days for presence of infection.

C. Examples

The examples presented below are provided as a further guide to the practitioner of ordinary skill in the art, and are not to be construed as limiting the invention in any way.

Example 1

(Demonstration of Activity)

A. Materials

Buffers were prepared as follows:

4×DHFR buffer: 200 mM Tes, 300 mM BME, 4 mM EDTA, pH 7.0.

+DHF buffer: 2.5 mg/mL BSA, 0.25 mM NADPH, 62.5 µM dihydrofolate, 2.5× DHFR buffer.

-DHF buffer: 2.5 mg/mL BSA, 0.25 mM NADPH, 2.5× DHFR buffer.

Enzyme buffer: 50 mM Tes, 5 mM DTT, 1 mM EDTA, 20% glycerol, 1 mg/mL BSA, pH 7.0.

Dilution buffer: 50 mM Tes, 5 mM DTT, 1 mM EDTA, 1 mg/mL BSA, pH 7.0.

PcDHFR: 5 µg/mL P. carinii DHFR in enzyme buffer.

crude hDHFR: crude recombinant human DHFR (obtained from Hoffmann-LaRoche) in enzyme buffer (9.9 mg/mL total protein).

purified hDHFR: purified recombinant human DHFR (obtained from Hoffmann-LaRoche) in enzyme buffer (3.5 mg/mL total protein).

Test compounds were prepared and provided by Hoffmann-LaRoche. Stock solutions were prepared by dissolving 2-8 mg in dimethylsulfoxide (DMSO)

to prepare 50 mM solutions. Compounds which did not dissolve at 50 mM were diluted serially to 25, 16.6, or 12.5 mM.

B. Assay

Eight compounds (6.7 µL each) were added to wells in one column of a Falcon® 96-well microtiter plate, and diluted with 160 µL of water. The remaining wells received 126 µL of water. The solutions in the first column were then serially diluted (4×) into columns 2-9, with column 10 containing a control sample, and columns 11-12 containing water blanks. The final compound concentrations were 1 mM to 15 nM (after the remaining reagents were added).

Using an 8-channel pipet, 100 µL of -DHF buffer were added to each well in columns 11 and 12 (blanks containing only water). Next, 100 µL of +DHF buffer were added to each well in columns 1-10. PcDHFR or crude or purified rhDHFR enzyme was then added (25 µL) to each well, and the plate contents mixed using a Titertek shaker. The plates were read on a Molecular Devices Plate Reader at an absorbance of 340 nm, -0.05 O.D. scale, for 10 minutes reading every 10 seconds. The kinetic data was analyzed using Delta Soft software (Biometallics), and the IC_{50} and Ki calculated for both human and P. carinii enzymes. Selectivity was calculated as $Ki_{(human)}/Ki_{(P.\ carinii)}$; thus, higher values for selectivity indicate that the compound inhibits the P. carinii enzyme to a greater degree than the human enzyme. The results are shown in Table 1 below. For purposes of comparison, trimethoprim in this assay exhibits an IC_{50} of 20 µM, with a selectivity of 0.1.

TABLE 1: Compounds of Formula I

R_3	R_4	R_5	IC_{50}	Selectivity
-OMe	-OMe	$-O(CH_2)_2NHSO_2\phi NHAc$	2.4 µM	350
-OMe	$-CMe_2OH$	-OMe	25 µM	350
-OEt	pyrrolyl	-OEt	2.4 µM	310
-OMe	pyrrolyl	-OMe	29 µM	30
vinyl	vinyloxy	vinyl	3.4 µM	250
H	BzO-	H	16 µM	50
-OMe	BzO-	BzO-	8.3 µM	100
-OMe	$-C(Me)=CH_2$	-OMe	24 µM	160
-OEt	$-C(Me)=CH_2$	-OEt	2.5 µM	160
-OMe	-SMe	-OMe	19 µM	145
-SMe	-OMe	-S(O)Me	18 µM	490
-OMe	Br	$-O(CH_2)_2CH(CH_2)_2COOH$ COOH	0.04 µM	525
-OMe	Br	$-O(CH_2)_2CO$(N-Asp)	2 µM	4300
H	-OMe	$-O(CH_2)_2CO$(N-Gly)	9.3 µM	80
-OMe	Br	$-O(CH_2)_2CO$(N-Gly)	10 µM	8

Me = $-CH_3$, Et = $-CH_2CH_3$, Bz = $-CH_2C_6H_6$, N-Gly = $-NHCH_2COOH$, N-Asp = $-NHCH(COOH)CH_2COOH$, ϕ = phenyl, Ac = $-COCH_3$

Example 2

Proceeding as described in Example 1 above, the compounds listed below were assayed and found to exhibit high activity and selectivity, as set forth in Table II:

A) 2,4-diamino-5-(4-benzyloxybenzyl)pyrimidine;

B) 2,4-diamino-5-(3,4-dimethoxy-5-benzyloxybenzyl)pyrimidine;

C) 2,4-diamino-5-(3,4-dibenzyloxy-5-methoxybenzyl)pyrimidine;

D) 2,4-diamino-5-[3,5-dimethoxy-4-(2-hydroxyprop-2-yl)benzyl]pyrimidine;

E) 2,4-diamino-5-(3,5-dimethoxy-4-N-pyrrolylbenzyl)pyrimidine;

F) 2,4-diamino-5-(3,5-diethoxy-4-N-pyrrolylbenzyl)pyrimidine;

G) 2,4-diamino-5-(3,5-divinyl-4-vinyloxybenzyl)pyrimidine;

H) 2,4-diamino-5-[3-(4-N-acetaminophenyl)sulfonaminoethoxy-4,5-dimethoxybenzyl]pyrimidine;

I) 2,4-diamino-5-[3-(4-aminophenyl)sulfonaminoethoxy-4-methoxybenzyl]-pyrimidine;

J) 2,4-diamino-5-[3-(4-N-acetaminophenyl)sulfonaminoethoxy-4-bromo-5-methoxybenzyl]pyrimidine;

K) 2,4-diamino-5-[3-(4-aminophenyl)sulfonaminoethoxy-4-bromo-5-methoxybenzyl]pyrimidine;

L) 2,4-diamino-5-[3,5-diethoxy-4-(propen-2-yl)benzyl]pyrimidine;

M) 2,4-diamino-5-[3,5-dimethoxy-4-(propen-2-yl)benzyl]pyrimidine;

N) 2,4-diamino-5-(3,5-dimethoxy-4-methylthiobenzyl)pyrimidine;

O) 2,4-diamino-5-(3-methylsulfinyl-4-methoxy-5-methylthiobenzyl)-pyrimidine;

P) 2,4-diamino-5-[3-(4,6-dicarboxyhexyloxy)-4-bromo-5-methoxybenzyl]-pyrimidine;

Q) 2,4-diamino-5-[3-(3-carboxymethylamino-3-oxopropoxy)-4-methoxybenzyl]pyrimidine;

R) 2,4-diamino-5-(3-[3-(1,3-dicarboxypropyl)amino-3-oxopropoxy]-4-bromo-5-methoxybenzyl)pyrimidine;

S) 2,4-diamino-5-[3,5-dimethoxy-4-((2-phenylsulfonyl)acetyl)benzyl]-pyrimidine;

T) 2,4-diamino-5-[3-amino-4-methyl-5-(N-pyrrolyl)benzyl]pyrimidine;

U) 2,4-diamino-5-(3,5-di-N-pyrrolyl-4-methoxybenzyl)pyrimidine;

V) 2,4-diamino-5-[3,5-di-methoxy-4-(3-hydrocarboxy-1-oxopropylamino)-benzyl]pyrimidine;

W) 2,4-diamino-5-[3,5-dimethoxy-(4-acetaminophenylsulfonamino)benzyl]-pyrimidine;

X) 2,4-diamino-5-(3,5-dimethoxy-4-propylbenzyl)pyrimidine;

Y) 2,4-diamino-5-(3,5-dichloro-4-N-pyrrolylbenzyl)pyrimidine;

Z) 2,4-diamino-5-[3,5-dimethoxy-4-(2-(2-(2-methoxy)ethoxy)ethoxy)ethoxy-benzyl]pyrimidine;

AA) 2,4-diamino-5-[3-(3-benzyloxycarbonylmethylamino-3-oxopropoxy)-4-bromo-5-methoxybenzyl]pyrimidine;

BB) 2,4-diamino-5-[3-(3-carboxymethylamino-3-oxopropoxy)-4-bromo-5-methoxybenzyl]pyrimidine;

CC) 2,4-diamino-5-[3-methoxy-4-bromo-5-(4-methylaminobenzamidoethoxy)-benzyl]pyrimidine;

DD) 3-(2,4-diaminopyrimidin-5-ylmethyl)-8-(3,5-dimethoxyphenyl)-8-aza-bicyclo[3.2.1]octane;

EE) 2H,3H-dihydro-5-(2,4-diaminopyrimidin-5-ylmethyl)-6,7-dimethoxybenzo-furan;

FF) 5-(2,4-diaminopyrimidin-5-ylmethyl)-7-methoxy-8-bromo-1,2-benzopyran;

GG) 5-(2,4-diaminopyrimidin-5-ylmethyl)-7,8-dimethoxy-1,2-benzopyran;

HH) 2,4-diamino-5-[3-phenyl-5-(3-methoxypropoxy)benzyl]pyrimidine;

II) 2,4-diamino-7-(3,5-dimethoxybenzyl)pyrrolo[2,3-f]quinazoline;

JJ) 2,4-diamino-5-[6-(4-methoxybutoxy)naphth-1-yl]pyrimidine;

KK) 2,4-diamino-5-(4,5,6-trimethoxy-2,3-dihydroinden-1-yl)pyrimidine;

LL) 2,2-dimethyl-5-(2,4-diaminopyrimidin-5-ylmethyl)-7-methoxybenz[b]di-oxolane;

MM) 2,4-diamino-5-(3,5-diethoxy-4-carboethoxybenzyl)pyrimidine; and

NN) 2,4-diamino-5-(2,7-dimethylbenzpyrazol-5-ylmethyl)pyrimidine.

TABLE II:

Compound	IC_{50}	Selectivity
A)	16.0	53.2
B)	13.9	3.3
C)	8.3	90.3
D)	24.8	343.5
E)	2.9	29.4
F)	2.4	312.4
G)	3.4	250.5
H)	2.4	248.5
I)	0.57	7.8
J)	3.7	29.5
K)	0.97	12.1
L)	2.5	190.9
M)	24.0	159.8
N)	19.0	143.5
O)	17.5	486.8
P)	0.043	528.0
Q)	9.3	77.7
R)	2.0	4255.0
S)	14.8	36.8
T)	33.5	42.5
U)	4.5	47.7
V)	100.0	85.2
W)	31.0	14.3
X)	20.0	42.6
Y)	10.9	53.2
Z)	36.0	33.1

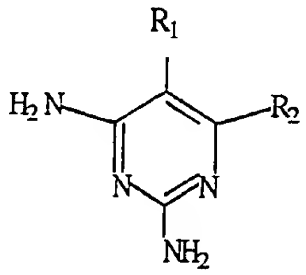
TABLE II (continued):

Compound	IC_{50}	Selectivity
AA)	7.9	14.4
BB)	10.0	13.1
CC)	7.8	14.6
DD)	0.016	31.0
EE)	12.9	26.4
FF)	5.9	6.9
GG)	11.5	266.7
HH)	12.2	11.2
II)	0.039	1.2
JJ)	80.0	106.5
KK)	87.0	97.9
LL)	13.9	15.0
MM)	8.3	63.6
NN)	18.0	10.7

What is Claimed:

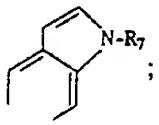
1. A method for treating fungal infection in a mammal, which method comprises:

administering to a mammal infected with a fungal pathogen an effective amount of a compound of formula I:



wherein

R_1 is 3-R_3-4-R_4-5-R_5-benzyl or (N-R_6)-8-azabicyclo[3.2.1]oct-3-yl;

and R_2 is H; or R_1 and R_2 together form  ;

where R_3 and R_5 are independently selected from the group consisting of H, lower alkoxy, lower alkylthio, lower alkylsulfinyl, vinyl, carboxy-lower alkyl, carboxy-lower alkoxy, dicarboxy-lower alkyl, dicarboxy-lower alkoxy, aryl-lower alkoxy, arylsulfonyl-lower alkoxy, arylsulfamido-lower alkoxy, and radicals of formula $-O(CH_2)_n-COR_8$, where n is an integer from 0 to 6 and R_8 is an amino acid;

R_4 is selected from the group consisting of lower alkoxy, aryl-lower alkoxy, lower alkylthio, halo, lower alkenyl, lower alkenyloxy, and pyrrolyl;

with the proviso that R_3, R_4, and R_5 are not simultaneously methoxy;

R_6 is selected from the group consisting of unsubstituted aryl and aryl substituted with one to three radicals selected from the group consisting of halo, lower alkyl, lower alkoxy, lower alkylthio, carboxy, carbamido, carboxy-lower alkyl, and carbamido-lower alkyl; and

R_7 is selected from the group consisting of aryl and aryl-lower alkyl, where aryl may be substituted with one to three radicals selected from the group consisting of halo, lower alkyl, lower alkoxy, lower alkylthio, carboxy, carbamido, carboxy-lower alkyl, and carbamido-lower alkyl;

and lower alkyl esters, amides thereof, and pharmaceutically acceptable addition salts.

2. The method of claim 1, wherein R_2 is H and R_1 is 3-R_3-4-R_4-5-R_5-benzyl.

3. The method of claim 2, wherein R_3 is methoxy.

4. The method of claim 3, wherein R_4 is bromo.

5. The method of claim 4, wherein R_5 is $-O(CH_2)_2CH(COOH)(CH_2)_2COOH$, or a pharmaceutically acceptable mono- or diester thereof.

6. The method of claim 4, wherein R_5 is $-O(CH_2)_n-COR_8$.

7. The method of claim 6, wherein n is 2 and R_8 is $-NHCH(COOH)CH_2CH_2COOH$ or a mono- or di-ester thereof.

8. The method of claim 6, wherein n is 2 and R_8 is $-NHCH_2COOH$ or an ester thereof.

9. The method of claim 3 wherein R_5 is methoxy.

10. The method of claim 9, wherein R_4 is 2-hydroxyprop-2-yl.

11. The method of claim 9, wherein R_4 is propen-2-yl.

12. The method of claim 9, wherein R_4 is N-pyrrolyl.

13. The method of claim 9, wherein R_4 is methylthio.

14. The method of claim 3, wherein R_4 is benzyloxy.

15. The method of claim 14, wherein R_5 is benzyloxy.

16. The method of claim 3, wherein R_4 is methoxy, and R_5 is $-OCH_2CH_2NHSO_2(C_6H_6)NHCOCH_3$.

17. The method of claim 2 wherein R_3 is H.

18. The method of claim 9 wherein R_4 is methoxy.

19. The method of claim 10 wherein R_5 is $-O(CH_2)_n-COR_8$, where n is 2 and R_8 is $-NHCH_2COOH$ or an ester thereof.

20. The method of claim 9, wherein R_4 is benzyloxy and R_5 is H.

21. The method of claim 2, where R_3 and R_5 are each ethoxy.

22. The method of claim 21, wherein R_4 is pyrrolyl.

23. The method of claim 21, wherein R_4 is propen-2-yl.

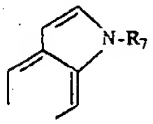
24. The method of claim 2, where R_3 and R_5 are each vinyl.

25. The method of claim 2, where R_3 is methylthio, R_4 is methoxy, and R_5 is methylsulfinyl.

26. The method of claim 1, wherein R_2 is H and R_1 is (N-R_6)-8-azabicyclo[3.2.1]oct-3-yl.

27. The method of claim 26, wherein R_6 is selected from the group consisting of 2-naphthyl, 3,5-dimethoxyphenyl, and 4-carboethoxyphenyl.

28. The method of claim 1, wherein R_1 and R_2 together form



29. The method of claim 28, wherein R_7 is benzyl.

30. The method of claim 28 where R_7 is 3,5-dimethoxyphenyl.

31. The method of claim 2 where R_3 and R_5 are propoxy and R_4 is N-pyrrolyl.

32. The method of claim 1 wherein said fungal pathogen is Pneumocystis carinii.

33. The method of claim 1, further comprising coadminstering a dihydropteroate synthase inhibitor.

34. The method of claim 33, wherein said dihydropteroate synthase inhibitor is selected from the group consisting of dapsone and sulfa drugs.

35. The method of claim 1, wherein said compound of formula I is selected from the group consisting of 2,4-diamino-5-(4-benzyloxy-benzyl)pyrimidine; 2,4-diamino-5-(3,4-dimethoxy-5-benzyloxybenzyl)pyrimidine; 2,4-diamino-5-(3,4-dibenzyloxy-5-methoxybenzyl)pyrimidine; 2,4-diamino-5-[3,5-dimethoxy-4-(2-hydroxyprop-2-yl)benzyl]pyrimidine; 2,4-diamino-5-(3,5-dimethoxy-4-N-pyrrolylbenzyl)pyrimidine; 2,4-diamino-5-(3,5-diethoxy-4-N-pyrrolylbenzyl)pyrimidine; 2,4-diamino-5-(3,5-divinyl-4-vinyloxybenzyl)-pyrimidine; 2,4-diamino-5-[3-(4-N-acetaminophenyl)sulfonaminoethoxy-4,5-dimethoxybenzyl]pyrimidine; 2,4-diamino-5-[3-(4-aminophenyl)sulfonaminoethoxy-4-methoxybenzyl]pyrimidine; 2,4-diamino-5-[3-(4-N-acetaminophenyl)sulfonaminoethoxy-4-bromo-5-methoxybenzyl]pyrimidine; 2,4-diamino-5-[3-(4-aminophenyl)sulfonaminoethoxy-4-bromo-5-methoxybenzyl]-pyrimidine; 2,4-diamino-5-[3,5-diethoxy-4-(propen-2-yl)benzyl]pyrimidine; 2,4-diamino-5-[3,5-dimethoxy-4-(propen-2-yl)benzyl]pyrimidine; 2,4-diamino-5-(3,5-dimethoxy-4-methylthiobenzyl)pyrimidine; 2,4-diamino-5-(3-methylsulfinyl-4-methoxy-5-methylthiobenzyl)pyrimidine; 2,4-diamino-5-[3-(4,6-dicarboxyhexyloxy)-4-bromo-5-methoxybenzyl]pyrimidine; 2,4-diamino-5-[3-(3-

carboxymethylamino-3-oxopropoxy)-4-methoxybenzyl]pyrimidine; 2,4-diamino-5-(3-[3-(1,3-dicarboxypropyl)amino-3-oxopropoxy]-4-bromo-5-methoxybenzyl)-pyrimidine; 2,4-diamino-5-[3,5-dimethoxy-4-((2-phenylsulfonyl)acetyl)benzyl]-pyrimidine; 2,4-diamino-5-[3-amino-4-methyl-5-(N-pyrrolyl)benzyl]pyrimidine; 2,4-diamino-5-(3,5-di-N-pyrrolyl-4-methoxybenzyl)pyrimidine; 2,4-diamino-5-[3,5-di-methoxy-4-(3-hydrocarboxy-1-oxopropylamino)benzyl]pyrimidine; 2,4-diamino-5-[3,5-dimethoxy-(4-acetaminophenylsulfonamino)benzyl]pyrimidine; 2,4-diamino-5-(3,5-dimethoxy-4-propylbenzyl)pyrimidine; 2,4-diamino-5-(3,5-dichloro-4-N-pyrrolylbenzyl)pyrimidine; 2,4-diamino-5-[3,5-dimethoxy-4-(2-(2-(2-methoxy)-ethoxy)ethoxy)ethoxybenzyl]pyrimidine; 2,4-diamino-5-[3-(3-benzyloxycarbonyl-methylamino-3-oxopropoxy)-4-bromo-5-methoxybenzyl]pyrimidine; 2,4-diamino-5-[3-(3-carboxymethylamino-3-oxopropoxy)-4-bromo-5-methoxybenzyl]pyrimidine; 2,4-diamino-5-[3-methoxy-4-bromo-5-(4-methylaminobenzamidoethoxy)benzyl]pyrimidine; 3-(2,4-diaminopyrimidin-5-ylmethyl)-8-(3,5-dimethoxyphenyl)-8-aza-bicyclo[3.2.1]octane; 2H,3H-dihydro-5-(2,4-diaminopyrimidin-5-ylmethyl)-6,7-dimethoxybenzofuran; 5-(2,4-diaminopyrimidin-5-ylmethyl)-7-methoxy-8-bromo-1,2-benzopyran; 5-(2,4-diaminopyrimidin-5-ylmethyl)-7,8-dimethoxy-1,2-benzopyran; 2,4-diamino-5-[3-phenyl-5-(3-methoxypropoxy)benzyl]pyrimidine; 2,4-diamino-7-(3,5-dimethoxybenzyl)pyrrolo[2,3-f]quinazoline; 2,4-diamino-5-[6-(4-methoxy-butoxy)naphth-1-yl]pyrimidine; 2,4-diamino-5-(4,5,6-trimethoxy-2,3-dihydroinden-1-yl)pyrimidine; 2,2-dimethyl-5-(2,4-diaminopyrimidin-5-ylmethyl)-7-methoxy-benz[b]dioxolane; 2,4-diamino-5-(3,5-diethoxy-4-carboethoxybenzyl)pyrimidine; and 2,4-diamino-5-(2,7-dimethylbenzpyrazol-5-ylmethyl)pyrimidine.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/08515

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 31/505;
US CL : 514/267; 514/269; 514/272; 514/275

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System [8]	Classification Symbols
U.S.	514/267, 514/269, 514/272, 514/275

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [9]

FUNGICIDAL, ANTIFUNGAL, PNEUMOCYSTIS CARINII, DIHYDROFOLATE REDUCTASE

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 3,852,450 (SILVESTRI ET AL.) 03 DECEMBER 1974 See entire document.	33-34
Y	US, A, 3,577,543 (BARANYCVITS ET AL.) 04 MAY 1971 See etire document.	1-35
Y	US, A, 4,374,136 (HILL ET AL.) 15 FEBRUARY 1983 See entire document.	1-35
Y	US, A, 4,640,923 (SCHWAINBORN ET AL.) 03 FEBRUARY 1987. See entire document.	1-35
Y,P	US, A, 4,996,198 (SCHILDKNECHT ET AL.) 26 FEBRUARY 1991. See entire document.	1-35
Y	EUROPE, A, 0139613 (HUBELE) 02 MAY 1985 See entire document.	1-35
Y	US, A, 4,954,498 (MERTENS ET AL.) 04 SEPTEMBER 1990 See entire document.	1-35
Y	US, A, 4,501,890 (NICHOLS ET AL.) 26 FEBRUARY 1985 See entire document.	1-35

* Special categories of cited documents: [14]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10 MARACH 1992	13 APR 1992
International Searching Authority	Signature of Authorized Officer
ISA/US	Russell Travers

Form PCT/ISA/210 (second sheet) (Rev. 11-87)

DC 3/12/92